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Localization, identification and characterization of a gut associated bacteria from piscine ectoparasite *Argulus bengalensis* Ramakrishna

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Abstract
Symbiotic bacteria are crucial for the survival of hematophagous arthropods, forming diverse relationships throughout evolutionary history. These bacteria typically exist in commensal or mutualistic associations. Various hematophagous arthropods house specific symbiotic bacteria in their gut. In a scanning electron microscopic study clusters of bacilli were observed in the gut of a haematophagus crustacean ectoparasite of fish *Argulus bengalensis*. The cultured colonies of the bacteria were isolated and subjected to biochemical characterization and identification through 16S rRNA sequencing. The study unveiled a Gram-negative bacillus different from *Acinetobacter baumannii* and *Aeromonas hydrophila*. Obtained in the earlier study. The strain was identified as *Aeromonas veronii* .This bacteria prefers acidic environments and is capable of metabolizing a broad spectrum of substrates to produce acid. Notably, *Aeromonas veronii* shows high sensitivity to norfloxacin, suggesting potential control methods for the parasite. *Aeromonas veronii*, commonly found in various environments, has increasingly been isolated from diseased fish exhibiting ulcers and fin rot, with *Argulus* parasites potentially facilitating its transmission through skin punctures. Symbiotic *Aeromonas veronii* in blood-feeding leeches synthesize B vitamins, aid in blood digestion, and inhibit other bacteria, suggesting a similar role in *Argulus.* These findings indicate possible symbiont replacements, evolutionary renewal, and high bacterial diversity in blood-feeding parasites.

Keywords: Piscine ectoparasite; *Argulus bengalensis*; symbiosis; *Aeromonas veronii*,

Introduction:

Organisms of the kingdom Animalia enjoy advantageous symbiotic relationships with microorganisms, primarily from the bacteria and archaea. Most of these associations are found in the gut of animals. Several relationships between the host-microbial interactions may be mutualistic, which implies that both the host and the microorganisms gain benefits that would be unattainable on their own.According to Trust and Sparrow (1974), Lindsay and Harris (1980), and Lesel et al. (1986), microbial communities are present in the digestive tracts of many invertebrates as well as vertebrates. These communities grow upon the food absorbed by the host animal and their digestive secretions. But do these microorganisms furnish in return to their hosts some useful substances coming from their metabolism? Are these microorganisms' only endo-commensals? The bacterial flora from endogenous sources possesses a significant and diverse enzymatic potential too. Our previous studies on gut flora of *Argulus bengalensis* demonstrated mutualistic associations of *Aeromonas hydrophila* and *Acinetobacter baumanii* with the parasitic host as both endosymbiont could release exoenzymes , anticoagulant chemicals and exhibit hemolytic activity enhancing the parasitic host's fitness thus promoting haematophagy.(Ghoshal;2023)

In continuation to our previous findings another colony of different appearance was observed in the culture plate which was subsequently characterised and identified In the present study.

Materials and methods:

Surface sterilization of parasites and isolation of the gut microflora:

In order to reduce the allochthonous gut flora and alimentary tract contents of ten adult *Argulus bengalensis* specimen were starved for four hours. It was followed by aseptically surface sterilization of the specimen using 0.01% mercuric chloride within a laminar flow hood. Under sterile conditions using an inverted microscope, dissection was performed to extract the gut tissues, and was homogenized aseptically. The homogenate was serially diluted in a 1:10 ratio, five times and used as inoculum as per Beveridge et al. (1991). Sterilized Tryptone Soya Agar (TSA) plates [pancreatic digest of caesin 15gL^{-1} papaic digest of soyabean 5 gL^{-1} sodium chloride 5 gL^{-1} agar agar 15 gL^{-1} were inoculated with 0.1 mL of each dilution in triplicate. To promote the growth of facultative anaerobic or aerobic bacterial colonies, the culture plates were incubated at 30°C for 24 hours. Pure cultures were obtained by streaking the well-separated colonies on TSA plates individually. Bacterial colonies were counted for the determination of CFU per mL. It was calculated by counting number of colonies **X** dilution of the supernatant **X** 10 / mL.

Growth parameters

Various abiotic growth factors, such as temperature, pH, and sodium chloride concentration, were explored to determine the optimal growth parameters for bacterial isolates.

Temperature Optimization

The bacterial isolates were inoculated into TSA media and their growth was measured varying temperature range of 10-55°C for 24 hours. Growth was assessed using a UV-spectrophotometer at 595 nm, and detailed observations were recorded.

pH Optimization

For pH optimization, the bacterial isolates were cultured in TSA media with a pH range of 4.0-10.0, followed by a 24-hour incubation at 30°C. Growth was evaluated using a UV-spectrophotometer at 595 nm, and thorough observations were documented.

NaCl Concentration Optimization

To determine the optimal NaCl concentration for growth, the bacterial isolates were introduced into TSA media with varying NaCl concentrations (2%-9 %). Following a 24-hour incubation at 30°C, growth was measured using a UV-spectrophotometer at 595 nm, and comprehensive observations were noted.

Biochemical characterization

According to Bergey's Manual of Determinative Biology (Holt et al., 1994), few key identifying biochemical characterizations were carried out.

Antibiotic Susceptibility Test

The antibiotic susceptibility of the bacterial isolates were assessed using the disc diffusion technique and susceptibility test discs from HiMedia, India, following the procedures outlined by the National Committee for Clinical Laboratory Standard (NCCLS, 2012). The inoculated plates are incubated at 37°C for 24 hrs. Different antibiotics viz. Doxycycline (30μg), Ampicillin (30μg), Chloramphenicol (30μg), Streptomycin (10 μg), Norfloxacin

(30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg) and Netilmicin (30µg) were used. Clear halo zones surrounding the discs indicated the susceptibility of the bacteria against the specific concentration of antibiotic.

Localization of gut microbiome

To study the localization of microbes in the gut of *Argulus bengalensis* scanning electron microscopy (SEM) of the gut was carried out. For the preparation of specimens for SEM, alive *Argulus* were subjected to pacification with gradual addition of ethanol followed by surface sterilization with 0.01% $HgCl_2$. Following this, the specimens were thoroughly washed in 0.1 M phosphate buffer (pH 7.4) and fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 37°C for 4 hours. Then the organisms were incised to cut open the gut and post fixed with 1% osmium tetroxide in phosphate buffered solution (pH 7.4). The sample was then dehydrated through a series of ethanol concentrations (30%, 50%, 70%, 90%, and 100%), followed by a sequence of graded alcohol-amyl acetate solutions at ratios of 4:1, 2:1, 1:1, 1:2, and 1:4. The specimens were ultimately placed in absolute amyl acetate for 30 minutes. Critical point drying (CPD) was performed using a HITACHI-HCP-2 critical point drier. The dried samples underwent gold coating with approximately 100 A° thickness using an IB 2 Ion coater and were then examined using a HITACHI-S-530 scanning electron microscope, operating at 15 KV.

Morphological characterization:Gram staining andscanning electron microscopy

The gram staining was carried out as per standard protocol.The surface topology of the isolate, was examined using Scanning Electron Microscopy (SEM).A sterilized glass cover was utilized for the growth of the bacterial isolates in multiwall plates containing LB medium (Nacl 10 gL^{-1} , yeast extract 5 gL^{-1} and agar agar 15 gL^{-1}) The cultures were then incubated at 37°C for 16 hours. A bacterial smear on a cover slip was treated with 2.5% glutaraldehyde. The cover slip was left in this solution for 1 hour at room temperature. Subsequently, the cover slip was washed twice with PBS. Dehydration of the samples was carried out using ethanol (40-100%) for progressive treatment. SEM observations were conducted using a JEOL JSM-6360, UK, with an accelerating voltage of 10 KV, and images were recorded at a magnification of 10 X. This methodology enabled a detailed examination of the surface characteristics of the isolated bacteria, offering valuable insights into their structural features at a microscopic level.

Molecular characterization and identification

DNA isolation of microbial isolate involved homogenizing bacterial culture with 1 ml of extraction buffer, transferring it to a 2 mL microfuge tube. Gentle mixing with an equal volume of phenol, chloroform, and isoamyl alcohol (25:24:1) was followed by centrifugation for 15 minutes at 14000 rpm. The supernatant, obtained after a second centrifugation using isoamyl alcohol (24:1) and mixed with an equal volume of chloroform, underwent precipitation for 15 minutes at room temperature with the addition of 0.1 mL of 3M sodium acetate pH 7.0 and 0.7 mL of isopropanol. The DNA pellet, washed twice with 70% ethanol and briefly with 100% ethanol, was dissolved in TE (Tris-Cl, 10 mM pH 8.0, EDTA 1 mM), followed by air drying. Addition of 5µl DNAse-free RNAse A (10$mgmL^{-1}$) removed RNA. For 16S rRNA gene sequencing, CM1MG1 and CM1MG2 isolates were chosen, and the 16S rRNA gene was PCR-amplified using primers 518F (5' CCAGCAGCCGGTAATACG 3') and 800R (5' TACCAGGGTATCTAATCC 3'). The approximately 1400 bp purified PCR product underwent sequencing. The obtained sequences were subjected to BLAST analysis, and phylogenetically related sequences were aligned and used for constructing a phylogenetic tree (neighbor-joining) with MEGA 10. Partial sequences of the 16S rRNA gene for the two isolates were deposited in the NCBI GenBank database to obtain accession numbers (Senthilraj et al., 2016)

Results

Isolation of the gut microflora

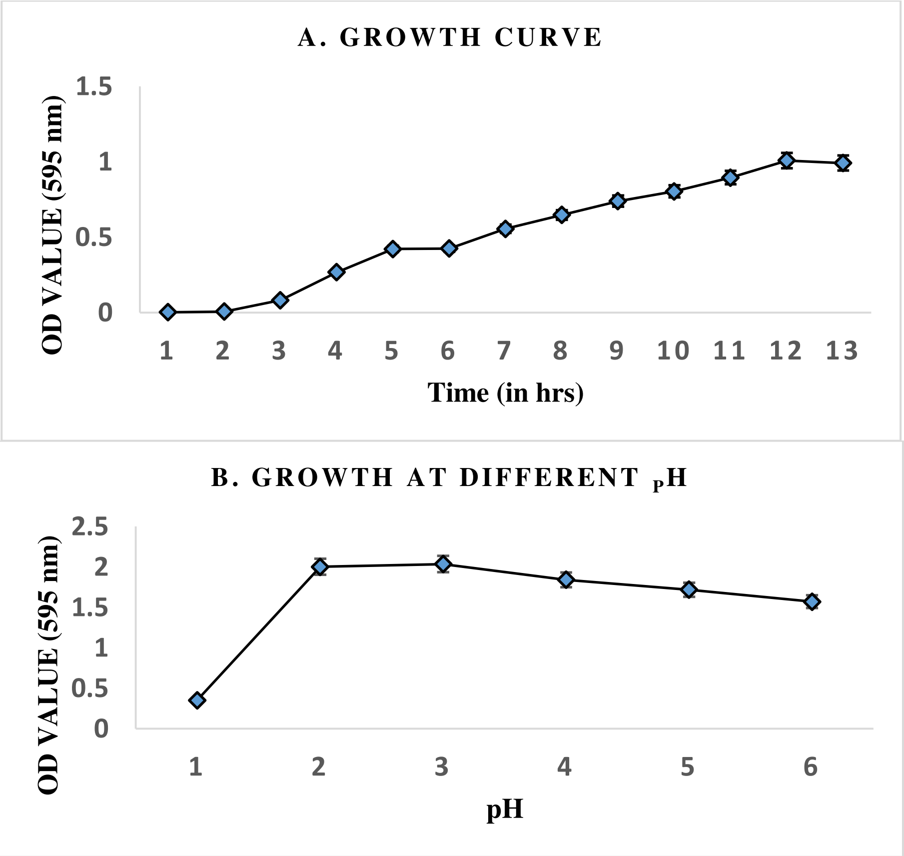
Upon incubation, only a single autochthonous bacterial colony was observed on the agar plate. This colony was distinguished by its morphology, encompassing features such as form, size, colour margin opacity, and elevation (**Table 1**). To generate pure cultures for subsequent analysis, the colony was selected for iterative subculture through streaking on a nutrient agar plate and was designated as FBRU-1 and further studies were carried out. The CFU was estimated as 10^{-3}/ mL

Table 1:Morphological characteristics of bacterial strain FBRU-1

CHARACTERISTICS	*FBRU1*
Colony morphology	Round
Colony elevation	Raised
Colony margin	Entire
Colony color	Yellowish white
Colony density	Opaque
Motility	Yes
Spore former	Yes
Gram staining	Negative

Growth parameters

The investigation into the growth performance of isolate FBRU-1 under diverse conditions unveiled that it manifests positive growth within a temperature spectrum of 20°C to 37°C. Following cultivation under varied pH conditions, FBRU-1 exhibited growth within the pH range of 5 to 9. Upon the study of growth of the isolate under various NaCl concentrations, it was observed that both isolates exhibited growth within the range of 1% to 4% NaCl concentration. Growth curve in different parameters are depicted in **Figure1**.



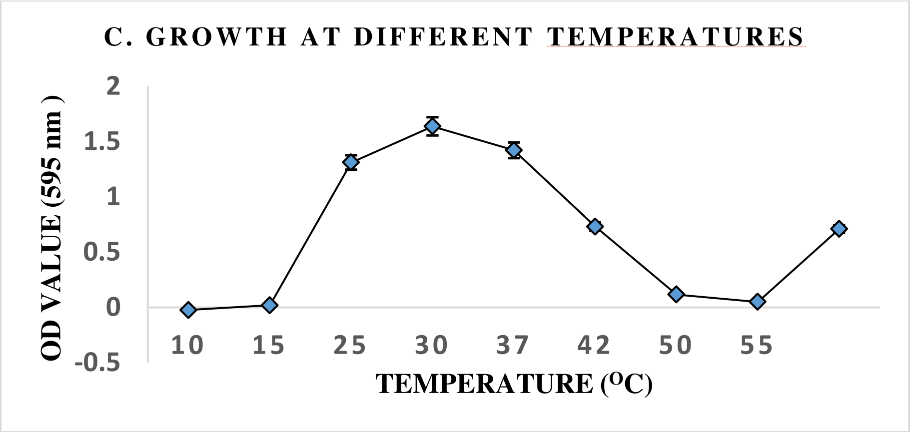

C. GROWTH AT DIFFERENT TEMPERATURES

FIGURE 1: Growing characteristics of FBRU1. The growth curve of FBRU1 at pH 7.0 and 37◦C (A) Growth at different pH (B) and temperature 10–42◦C (C). exhibited four distinct phases of bacterial growth (C).

Biochemical characteristics

The results of the biochemical tests revealed that the isolate FBRU-1 exhibited motility and hydrolyzed urea. It tested positive for maltose utilization, the Voges-Proskauer reaction, and produced acid from maltose, arabitol, mannose, glucose, sucrose, lactose, and fructose. However, it tested negative for raffinose, xylose etc. The details are mentioned in **Table 2.**

Table 2: Biochemical characteristics of FBRU1

Characteristics		**Acid production from**	
Esculine	+	**Maltose**	+
Urea	+	**Mannose**	+
VogesProskauer test	+	**Arabitol**	+
Indole	+	**Fructose**	+
H_2S Production	-	**Glucose**	+
Growth on NaCl		**Lactose**	+
1%	+	**Sucrose**	+
2%	+	**Raffinose**	-
3%	+	**Xylose**	-
4%	+	**Citrate**	-
Hemolytic	+	**Salicin**	-

Antibiotic Susceptibility Test

The isolate was found to be most susceptible to norfloxacin and moderately susceptible to doxycycline and netilmicin. The detailed results are enlisted in **Table 3.**

Table 3: Antibiotic sensitivity test of FBRU-1

Chemical	DiscContent (µg)	FBRU-1
Chloramphenicol	30	S
Streptomycin	10	R
Ampicillin	30	S
Netilmicin	30	MS
Ciprofloxacin	5	S
Doxycycline	30	MS
Cotrimoxazole	25	S
Norfloxacin	30	S

S=susceptible,M=moderatelysusceptible,R= Resistant

Localization

The scanning electron microscopy of the *Argulus* gut (Fig2) reveals colonization of multiple bacilli across various sections of the digestive tract of *Argulus.*

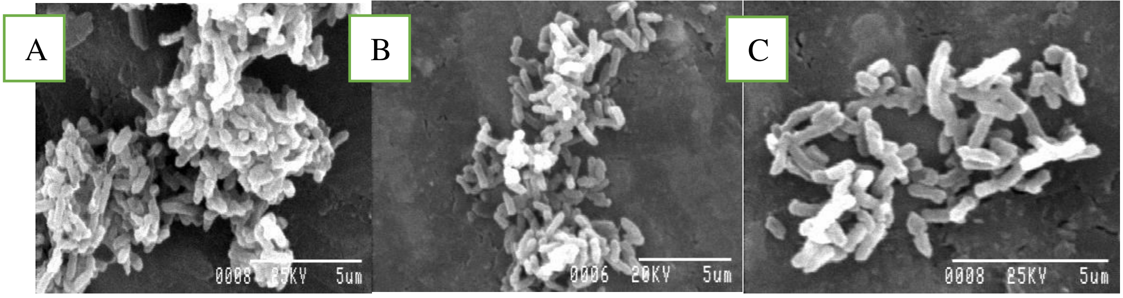


Figure 2: Scanning electron micrograph of gut of *Argulus* showing localisation of multiple clustered bacilli (A, B and C)

Morphological characteristics through SEM

Scanning electron micrographs of FBRU-1 shows the surface topology and it was observed that it is bacillus in structure (Fig 3)

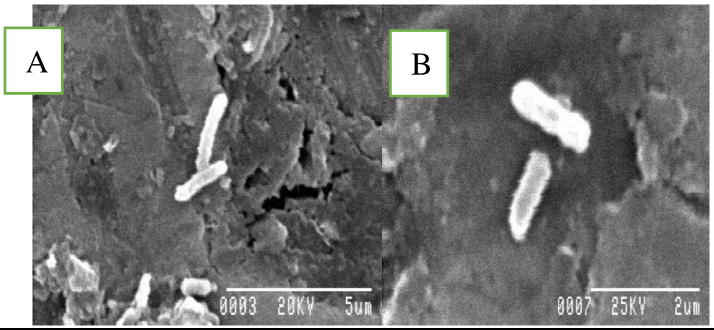


Figure 3: Scanning electron micrograph of surface topology of FBRU1 (A and B)

Molecular Characterization

The result of the BLAST analysis for FBRU-1 is shown in **table 4**

Table 4: BLAST analysis report for FBRU-1

Short ID	S_ab Score	Unique common oligomers	Sequence full name
S000142447	0.992	1413	*Aeromonas* sp.; ATCC 35941; X60417
S000428862	0.992	1434	*Aeromonas veronii*; M16; AF099023
S000428863	0.992	1434	*Aeromonas veronii*; B1; AF099024
S000614573	0.992	1424	*Aeromonas* sp. m22; DQ219814
S000775565	0.992	1420	*Aeromonas veronii*; AE-41; AY987729
S000775584	0.992	1420	*Aeromonas veronii*; CECT 4246; AY987748
S000775601	0.992	1423	*Aeromonas* sp. RK 70363; AY987765

Through an evolutionary analysis of the 16S rRNA gene sequence and nucleotide sequence homology, it was determined that strain was similar with the type strains of *Aeromonas veronii*. The type strains were sourced from the NCBI Gen Bank. Dendrograms in **Figure 3** illustrate its evolutionary relationships with their closely related type strains, retrieved from the NCBI GenBank.

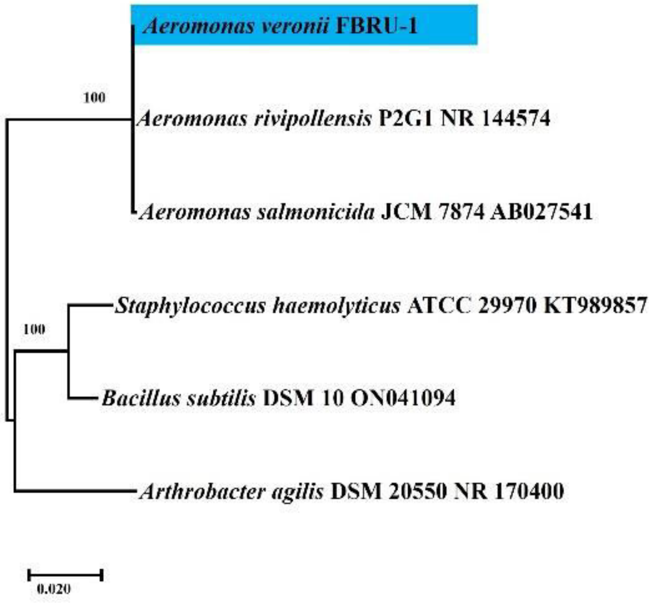


Figure 4:NCBI algorithm based phylogenetic tree of FBRU-1 isolated from *Argulus bengalensis* gut.

Discussion

The existence of symbiotic bacteria is essential for survival of hematophagous arthropods. Bacteria and arthropods have forged a diverse range of symbiotic partnerships throughout their evolutionary histories (Heddi and Gross, 2012; Nation, 2016). Within arthropods, the majority of these bacterial species exist in commensal or mutualistic relationships (Douglas, 2015; Heddi and Gross, 2012). Haematophagus parasites of vertebrates typically develop unique microhabitat for housing particular symbiotic bacteria through their life stages: Tsetse fly *Glossina* sp. symbioses with *Wigglesworthia* (Aksoy, 1995; Akman et al., 2002)) *Wolbachia* with bedbugs (Hosokawa et al., 2010; Nikoh et al., 2014), *Aschnera* with nycteribiid bat flies (Hosokawa et al., 2012), etc. *Argulusbengalensis* is a crustacean ectoparasite of fish which is also haematophagus in nature. Scanning electron microscopy results (Fig.2) clearly demonstrate that clusters of bacilli are localized in various regions of the gut of *Argulus bengalensis*. Since the organisms were starved for almost 4 hrs before the study, it is reasonable to infer that these isolates are regarded as integral components of the autochthonous adherent microflora of *Argulus* aligning with the assertions made by Ghosh et al. (2010) in carps. Comparable instances have been documented by Gross (2002) in *Camponotus floridanus* carpenter ants, where the distribution of endosymbiotic bacteria across different tissues of queens, males, and workers was analysed using light and electron microscopy, as well as in situ hybridization. Additionally, due to its naturally limited microbiome, dominated by two primary microbial components within the alimentary canal, the Hungarian medicinal leech, *Hirudo verbana*, serves as an intriguing model for studying host-microbe symbioses (Graf, 2006). Our earlier research on the gut-associated microbiota of *Argulus bengalensis* identified a mutualistic relationship between *Acinetobacter baumannii* and *Aeromonas hydrophila* (Ghoshal, 2023). The current study further reveals the presence of an additional Gram-negative bacillus in the gut, distinguished by slight variations in colony morphology and chemical characteristics compared to that of the previously identified species. Upon molecular chararecterisation by 16sr RNA sequencing the isolate was identified to be a type strain of *Aeromonas veronii* M16; AF099023. Which are closely related to type strain of with *Aeromonas rivipollensis* and *Aeromonas salmonicida*

The microbial colonization within various gut compartments is influenced by physicochemical conditions, exhibiting significant fluctuations in both pH and oxygen levels. Both *Acinetobacter baumannii* and *Aeromonas hydrophila* shows their ability to survive at a wide pH range from pH 5 to 10 whereas this *Aeromonas veronii* strain prefers acidic environment and grows within a range of pH1 to 4. As far as acid production is concerned all three identified gut bacteria from *Argulus* are capable of metabolizing a broad spectrum of substrates to produce acid. Moreover, the bacteria is highly sensitive to norfloxacin which may be put on trial against the symbiont to control the parasite.

Originally described by Hickman-Brenner et al. (1987), *Aeromonas veronii* is a Gram-negative, rod-shaped bacteria commonly found in environmental, clinical, and food samples (Ghenghesh et al., 2008). There has been a rising incidence of *A. veronii* strains isolated from diseased fish (Hoai et al., 2019; Raj et al., 2019). Infected fish generally exhibit clinical symptoms such as ulcers, fin rot/tail rot, abdominal distention, exophthalmia, and). So it is haemorrhaging. Multiple puncturing of the fish skin by argulid parasites (Banerjee et al; 2011)alsoresults in secondary infections (Raman; 2021) and the fact is relevant to explain transmission of the pathogenic Aeromonas *veronii* to the fish host through blood feeding.

Findings of Perkins et al (unpublished data) suggest that blood-feeding leeches have established bacterial symbiotic partnerships with *Aeromonas veronii* throughout their evolutionary history. Numerous hypotheses have been proposed regarding the role of *Aeromonas* bacteria in medicinal leeches, suggesting that they not only synthesize B vitamins but also assist in blood digestion and inhibit the growth of other bacterial species in the digestive tract (Graf, 2000). It is thus presumed that *A. veronii* plays similar role in argulid host along with other two symbionts *Acinetobacter baumannii* and *Aeromonas hydrophila*. *Aeromonas* species have also been detected in the midgut of mosquito vectors, where they play an essential role in the mosquito's survival (Ding et al; 2024). It could thus be suggested that these results point to the potential for repeated symbiont replacements, with bacterial partnerships being renewed and improved over evolutionary time. This could also account for the phylogenetic diversity of primary symbionts among closely related arthropods. Similarly, argulids may acquire transient bacterial species that initially act as secondary symbionts but eventually assume essential, primary roles. This process could explain the unexpectedly high diversity of bacterial partners in blood-feeding parasitic hosts.

Conclusion:

The study elucidated the complex microbial interactions within the gut of the hematophagous crustacean *Argulus bengalensis*, highlighting the presence of *Aeromonas veronii* alongside previously identified mutualists, *Acinetobacter baumannii* and *Aeromonas hydrophila*. The identification of *A. veronii* as a key component of the autochthonous microflora underscores its role in the symbiotic relationship with *Argulus*, potentially aiding in digestion and providing a competitive advantage against other bacterial species. Our findings align with previous research on symbiotic bacteria in certain other haematophagus hosts, suggesting that leeches and *Argulus* may exhibit evolutionary patterns of repeated symbiont replacements, which contribute to the observed microbial diversity. The ability of these bacteria to adapt to varying environmental conditions, coupled with their metabolic capabilities, highlights their importance in the host's survival and presents potential avenues for controlling parasitic infections through targeted antimicrobial strategies.

References

Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., & Aksoy, S. (2002). Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia. Nature genetics*, *32*(3), 402-407.

Aksoy, S. (1995). *Wigglesworthia* gen. nov. and *Wigglesworthia glossinidia* sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. *International Journal of Systematic and Evolutionary Microbiology*, *45*(4), 848-851.

Bergey, D. H., & GH, J. (1994). Bergey'sManual of Determinative Biology. *Chapter*, *4*, 181-186.

Ghenghesh, K. S., Ahmed, S. F., El-Khalek, R. A., Al-Gendy, A., & Klena, J. (2008). *Aeromonas*-associated infections in developing countries. *The Journal of Infection in Developing Countries*, *2*(02), 081-098.

Ghoshal, M. ., Ghosh, K. ., & Saha, S. K. . (2023). Isolation, Characterization and Identification of Gut Bacteria from Piscine Ectoparasite *Argulus bengalensis* (Ramakrishna 1951) and their Relationship with Haematophagy. *Journal of Advanced Zoology*, *44*(2), 240–250

Heddi, A., & Gross, R. (2011). Proteobacteria as primary endosymbionts of arthropods. *Manipulative tenants, Bacteria associated with Arthropods. CRC Press, Boca Raton*, 1-27.

Hickman-Brenner, F. W., MacDonald, K. L., Steigerwalt, A. G., Fanning, G. R., Brenner, D. J., & Farmer 3rd, J. J. (1987). *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea. *Journal of clinical microbiology*, *25*(5), 900-906.

Hoai, T. D., Trang, T. T., Van Tuyen, N., Giang, N. T. H., & Van Van, K. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture*, *513*, 734425.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y., & Fukatsu, T. (2010). *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences*, *107*(2), 769-774.

Indergand, S., & Graf, J. (2000). Ingested blood contributes to the specificity of the symbiosis of *Aeromonas veronii Biovar sobria* and *Hirudo medicinalis*, the medicinal leech. *Applied and environmental microbiology*, *66*(11), 4735-4741.

Lesel, R., Fromageot, C., & Lesel, M. (1986). Cellulose digestibility in grass carp, *Ctenopharyngodon idella* and in goldfish, *Carassius auratus*. *Aquaculture*, *54*(1-2), 11-17.

Li, J., Du, J., Ding, G., Zhang, W., Zhou, Y., Xu, Y., ... & Shen, B. (2024). Isolation, characterization and functional analysis of a bacteriophage targeting *Culex pipiens* pallens resistance-associated *Aeromonas hydrophila*. *Parasites & Vectors*, *17*(1), 222.

Lindsay, G. J. H., & Harris, J. E. (1980). Carboxymethylcellulase activity in the digestive tracts of fish. *Journal of fish Biology*, *16*(3), 219-233.

Nation Sr, J. L. (2022). *Insect physiology and biochemistry*. CRC press.

Perkins, S. L., Budinoff, R. B., & Siddall, M. E. Bacterial Endosymbionts of Leeches.

Raj, N. S., Swaminathan, T. R., Dharmaratnam, A., Raja, S. A., Ramraj, D., & Lal, K. K. (2019). *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. *Aquaculture*, *512*, 734278.

Saha, S. K., Guha, A., & Banerjee, A. (2011). Feeding Apparatus and Associated Glands in the Freshwater Fish Ectoparasite *Argulus siamensis* Wilson, 1926 (Branchiura). *Crustaceana*, *84*(10).

Sauer, C., Dudaczek, D., Hölldobler, B., & Gross, R. (2002). Tissue localization of the endosymbiotic bacterium "*Candidatus Blochmannia floridanus*" in adults and larvae of the carpenter ant *Camponotus floridanus*. *Applied and Environmental Microbiology*, *68*(9), 4187-4193.

Shameena, S. S., Kumar, K., Kumar, S., Kumari, P., Krishnan, R., Karmakar, S., ... & Raman, R. P. (2021). Dose-dependent co-infection of *Argulus* sp. and *Aeromonas hydrophila* in goldfish (*Carassius auratus)* modulates innate immune response and antioxidative stress enzymes. *Fish & Shellfish Immunology*, *114*, 199-206.

Trust, T. J., & Sparrow, R. A. H. (1974). The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Canadian Journal of Microbiology*, *20*(9), 1219-1228.